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Phytochemical Study of a Species with Ethnopharmacological Interest: *Sideritis romana* L.

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Authors' contributions

This work was carried out in collaboration between all authors which contributed equally to this study. All authors read and approved the final manuscript.

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ABSTRACT

The phytochemical analysis of *Sideritis romana* L., a species largely used as a traditional remedy, led to the isolation and the identification of several acetylated flavonoid glycosides with apigenin, luteolin, hypolaetin and isoscutellarein backbone. Among these, an apigenin derivative (**2**) was recognized for the first time in *Sideritis* as well as in the *Lamiaceae* family. The iridoidic pattern of this species showed the presence of harpagide, currently considered the main taxonomical maker in this genus, together with 6-deoxyharpagide which is a rare compound since it was previously recognized in a limited number of species of *Lamiaceae*. Other identified iridoids were ajugoside and bartsioside, the latter recognized for the first time in the species as well as in the family. Acetylated flavonoid glycosides and iridoid glucosides are considered of chemotaxonomic relevance in several genera comprised in the Lamioideae subfamily and the occurrence of both of these classes of compounds in *S. romana* was discussed. The co-presence of the acetylated

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apigenin derivative and of 6-deoxyharpagide could be used as a marker at a specific level seen their relative rarity in *Lamiaceae*. Regarding the traditional uses of this species, the isolated compounds may give a rationale from a chemical standpoint.

Keywords: Sideritis romana; Lamiaceae; iridoids; flavonoids; chemotaxonomy; traditional medicine.

1. INTRODUCTION

Sideritis romana L., also known as ironwort, is an annual plant belonging to *Lamiaceae* family (Lamioideae subfamily). It is characterized by an erected stem which is simple or branched at the base. It owns elliptic basal leaves, the upper ones with a lanceolate shape. The flowers are inserted in outdistanced verticillasters and the youngest ones fill the inferior part of the inflorescence. The corolla is yellow with white or pink shades. The fruits are brown trigonal and warty nuculae. It can be found in the Mediterranean area where it widely grows in arid meadows and grazings till the altitude of 1900 m a.s.l. In Italy it is present in almost all the territory except for some regions of northern Italy like Veneto, Trentino Alto Adige and Valle d'Aosta and also in Piedmont where its presence is actually unsure [1]. It can be mistaken for *Stachys ocymastrum* (L.) Briq. but the latter owns leaves with different shape (oval and crenate along the margins excluding the base) and the youngest flowers fill the superior part of the inflorescence [2]. Many species of the genus *Sideritis* (mountain tea) are widely used in traditional medicine in the Mediterranean and Balkan regions as a tonic and for the treatment of several diseases i.e. gastrointestinal complaints, inflammations, and rheumatic disorders [3]. *Sideritis* are used in Turkish folk medicine as beverage in brew form for its high antibacterial, carminative, diuretic and digestive properties [4]. They are also used as anti-inflammatory, anti-ulcer, cytostatic, antimicrobial, vulnerary, astringent and circulation stimulating agents [5]. The genus name *Sideritis* is derived from the Greek word "sideros" (Σίδερος, iron) and was known to ancient Greeks because of its property to heal wounds caused by iron weapons during battles. In Italy, it is used for its vulnerary properties to heal cuts and wounds of the skin by the shepherds of the Madonie Mountains (Sicily) [6]. Moreover in some Italian regions, the decoy of the exsiccated leaves is used to wash the unconcealed parts of the body in case of fright in order to soothe anxiety [1].

In literature there are little studies on this species focusing mainly on the content of the essential oil [7,8]. Only particularities of the composition of the polar fraction are reported, i.e the revision of siderin structure [9] and the identification of a new glycosidic flavonoid [10]. There is also in literature a detailed review on *Sideritis* genus and its traditional uses [3] but the species *romana* obviously was not included since a complete phytochemical analysis of the polar fraction was not available. We have then decided to re-study a sample of this species, wanting to build a first more detailed and total molecular pattern on the polar fraction content.

2. MATERIALS AND METHODS

2.1 General

NMR spectra were recorded on a Varian (now Agilent Technologies) Mercury 300 MHz instrument and on a Bruker Avance III 400 MHz instrument, using $CDCl_3$, CD_3OD or D_2O as deuterated solvents. The chemical shifts are expressed in ppm from TMS in deuterochloroform; the signal of HDO (*s*) at 4.78 ppm was used as reference for spectra in D_2O; and the internal solvent signal (*m*5) at 3.31 ppm for spectra recorded in CD_3OD.

MS spectra were performed on a Q-TOF MICRO spectrometer (Waters, Manchester, UK) equipped with an ESI source operating in negative and/or positive ion mode. The flow rate of the sample infusion was 10 µl per min. with 100 acquisitions per spectrum. Data were analysed by the MassLynx software developed by Waters.

Solvents of RPE grade were purchased from Sigma Aldrich (Milan, Italy) or Carlo Erba Reagenti (Milan, Italy) and silica gel 60 (70-230 mesh ASTM) was from Fluka.

2.2 Bidimensional NMR Experiments

Bidimensional spectra were performed on a Bruker Avance III 400 MHz instrument, operating at 9.4 T at 298° K. NOESY experiments were acquired with a spectral width of 15 ppm in both dimensions, a mixing time length of 700 ms, recycle delay of 2 s and a data matrix of 4K x 256 points. HSQC experiments were acquired

with a spectral width of 15 and 250 ppm for the proton and carbon respectively, an average $^{1}J_{C\text{-}H}$ of 145 Hz, recycle delay of 2 s and a data matrix of 4K x 256 points. HMBC experiments were acquired with a spectral width of 15 and 250 ppm for the proton and carbon respectively, long range coupling constant of $^{n}J_{C\text{-}H}$ of 8 Hz, recycle delay of 2 s and a data matrix of 4K x 256 points.

2.3 Plant Material

A sample of fresh plant material (168.9 g) was harvested at flowering stage at the end of June 2014 in Central Italy on Latium hills at an altitude of 150 m a.s.l. (GPRS coordinates 41.615434, 13.548094). The botanical identification was performed by one of us (A.V.) using available literature [1,2]. The specimen of the studied plant is registered at "Museo Erbario, Dipartimento di Biologia Ambientale, Sapienza Università di Roma" under the following accession number: RO H.A. N°18419.

2.4 Extraction and Isolation of Polar Compounds

The dried plant material (168.9 g) was exhaustively extracted four times using 96% ethanol (0.3 L each, for 48 h). The extracts were collected altogether and the ethanol was eliminated at reduced pressure until a water suspension was obtained. This suspension was frozen and then lyophilized, to preserve also temperature-sensitive compounds, obtaining 15.3 g of crude extract.

Repeated chromatographic separation on silica gel column were conducted on a fraction of the crude extract (5.15 g), using different eluting systems: *n*-butanol saturated with water for the first separation and then several mixtures of chloroform/methanol at various percentages, also increasing the polarity during the chromatographic run, on specific fractions derived from the first chromatographic run.

From these separation procedures twelve compounds were isolated: (*E*)-phytol (**1**) (14.9 mg) [11,12], apigenin 7-*O*-(6''-*O*-acetyl-β-D-glucopyranoside) (**2**) (15.6 mg) [13], 7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (**3**) (39.7 mg), 7-{[2-*O*-(6-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy}-2-(3,4-dihydroxyphenyl)-5-hydroxy-4*H*-1-benzopyran-4-one (**4**) (11.9 mg), 7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(4-hydroxyphenyl)- 4*H*-1-benzopyran-4-one (**5**) (5.0 mg) [14], 7-{[2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one (**6**) (4.7 mg) [15], 7-{[6-*O*-acetyl-2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5,8-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (**7**) (5.1 mg), 7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5,8-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (**8**) (5.5 mg) [16], 6-deoxiharpagide (**9**) (7.0 mg) [17], harpagide (**10**) (10.2 mg) [18], ajugoside (**11**) (3.7 mg) [19] and bartsioside (**12**) (2.1 mg) [20] (Fig. 1).

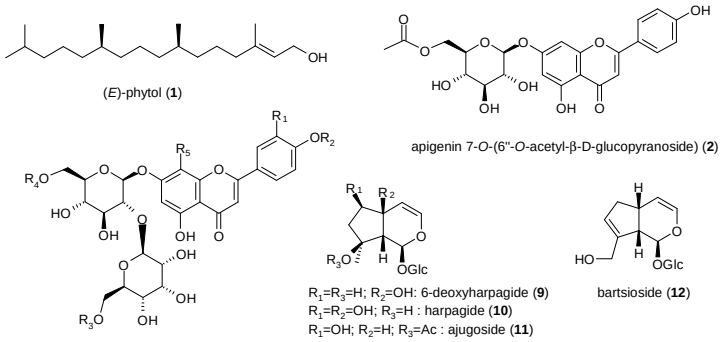
NMR data of compounds **1** and **9**-**11** were in accordance with literature data.

2.5 Spectral Data of Isolated Compounds

apigenin 7-*O*-(6''-*O*-acetyl-β-D-glucopyranoside) (**2**), ^{1}H-NMR (CD_3OD, 400 MHz), δ: 7.86 (2H, d, *J* = 8.8 Hz, H-2'/H-6'), 6.93 (2H, d, *J* = 8.8 Hz, H-3'/H-5'), 6.76 (1H, d, *J* = 2.1 Hz, H-8), 6.64 (1H, s, H-3), 6.49 (1H, d, *J* = 2.1 Hz, H-6), 5.04 (1H, d, *J* = 7.3 Hz, H-1''), 4.46 (1H, dd, *J* = 12.0, 2.2 Hz, H-6''a), 4.23 (1H, dd, *J* = 12.0, 7.1 Hz, H-6''b), 2.06 (3H, s, $\underline{CH_3}$CO).

^{13}C NMR (100 MHz, MeOD) δ: 184.1 (C-4), 172.7 ($CH_3\underline{CO}$), 166.7 (C-2), 164.6 (C-7), 162.9 (C-5), 158.9 (C-9), 129.6 (C-2'/C-6'), 123.1 (C-1'), 117.1 (C-3'/C-5'), 104.9 (C-10), 104.2 (C-3), 101.5 (C-1''), 101.1 (C-8), 96.2 (C-6), 77.8 (C-3''), 75.6 (C-5''), 74.7 (C-2''), 71.6 (C-4''), 64.8 (C-6''), 20.8 ($\underline{CH_3}$CO). ESI-MS: *m/z* 496.97 $[M+Na]^+$; *m/z* 472.92 $[M-H]^-$.

7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (3), ^{1}H NMR (400 MHz, DMSO) δ: 7.58 (2H, m, H-2'/H-6'), 6.98 (1H, s, H-3), 6.94 (1H, d, *J* = 8.9 Hz, H-5'), 6.80 (1H, d, *J* = 1.8 Hz, H-6), 6.45 (1H, d, *J* = 1.8 Hz, H-8), 5.21 (1H, d, *J* = 7.5 Hz, H-1''), 4.79 (1H, d, *J* = 7.9 Hz, H-1'''), 4.12 (1H, dd, *J* = 11.7, 1.8 Hz, H-6'''a), 4.00 (1H, dd, *J* = 11.7, 5.4 Hz, H-6'''b), 3.89 (3H, s, O$\underline{CH_3}$), 1.94 (3H, s, $\underline{CH_3}$CO).
ESI-MS: *m/z* 689.11 $[M+Na]^+$; *m/z* 665.02 $[M-H]^-$.



R_1=OH; R_2=CH_3; R_3=Ac; R_4=H; R_5=H : 7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (**3**)
R_1=OH; R_2=H; R_3=Ac; R_4=H; R_5=H : (7-{[2-*O*-(6-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy}-2-(3,4-dihydroxyphenyl)-5-hydroxy-4*H*-1-benzopyran-4-one (**4**)
R_1=H; R_2=H; R_3=Ac; R_4=H; R_5=H : 7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (**5**)
R_1=OCH_3; R_2=R_4=R_5=H : 7-[(2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl)oxy]-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one (**6**)
R_1=R_5=OH;R_2=H; R_3=R_4=Ac : 7-[[6-*O*-acetyl-2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy]-5,8-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (**7**)
R_1=R_2=R_4=H; R_5=OH; R_3=Ac : 7-[[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy]-5,8-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (**8**)

Fig. 1. Structures of compounds isolated from *Sideritis romana*

7-{[2-*O*-(6-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy}-2-(3,4-dihydroxyphenyl)-5-hydroxy-4*H*-1-benzopyran-4-one (**4**), ^{1}H-NMR (CD_3OD, 300MHz) δ**:** 7.61 – 7.52 (1H, br s, H-2'), 7.52 (1H, br d, *J* = 8.5 Hz, H-6'), 6.94 (1H, d, *J* = 8.4 Hz, H-5'), 6.83 (1H, d, *J* = 1.9 Hz, H-6), 6.70 (1H, s, H-3), 6.51 (1H, d, *J* = 1.9 Hz, H-8), 5.24 (1H, d, *J* = 6.7 Hz, H-1''), 4.98 (1H, d, *J* = 8.0 Hz, H-1'''), 4.16 (1H, dd, *J* = 12.2, 1.7 Hz, H-6''a), 2.06 (3H, s, $\underline{CH_3}$CO).
ESI-MS: *m/z* 675.10 $[M+Na]^+$; *m/z* 650.99 $[M-H]^-$.

7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-benzopyran-4-one (5), ^{1}H-NMR (CD_3OD, 300Hz) δ: 7.98 (2H, d, *J* = 8.2 Hz, H-2'/H-6'), 6.94 (1H, s, H-3), 6.92 (2H, d, *J* = 8.2 Hz, H-3'/H-5'), 6.73 (1H, d, *J* = 2.2 Hz, H-6), 6.42 (1H, d, *J* = 2.1 Hz, H-8), 5.18 (1H, d, *J* = 7.5 Hz, H-1''), 4.80 (1H, d, *J* = 7.9 Hz, H-1'''), 4.12 (1H, dd, *J* = 11.8, 1.9 Hz, H-6'''a), 4.00 (1H, dd, *J* = 11.8, 5.7 Hz, H-6'''b), 1.89 (3H, s, $\underline{CH_3}$CO).
ESI-MS: *m/z* 659.12 $[M+Na]^+$; *m/z* 635.02 $[M-H]^-$.

7-{[2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one (**6**), ^{1}H NMR (400 MHz, MeOD) δ: 7.57 (1H, dd, J = 8.5, 2.1 Hz, H-6'), 7.54 (1H, d, J = 2.1 Hz, H-2'), 6.95 (1H, d, J = 8.5 Hz, H-5'), 6.89 (1H, d, J = 2.2 Hz, H-6), 6.71 (1H, s, H-3), 6.53 (1H, d, J = 2.2 Hz, H-8), 5.27 (1H, d, J = 7.2 Hz, H-1''), 5.01 (1H, d, J = 7.8 Hz, H-1'''), 3.98 (3H, s, 3'-CH_3O), other sugar signals between 3.98 and 3.25 ppm.
ESI-MS: *m/z* 646.67 $[M+Na]^+$; *m/z* 622.85 $[M-H]^-$.

7-{[6-*O*-acetyl-2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5,8-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (**7**), ^{1}H NMR (400 MHz, MeOD) δ: 7.56 (1H, br d, J = 8.9 Hz, H-6'), 7.55 (1H, br s, H-2'), 6.96 (1H, d, J = 8.6 Hz, H-5), 6.84 (1H, br s, H-6), 6.53 (1H, s, H-3), 5.25 (1H, d, J = 7.8 Hz, H-1''), 5.00 (1H, d, J = 8.0 Hz, H-1'''), 3.99 (3H, s, 4'-CH_3O), 2.01 (3H, s, $\underline{CH_3}$CO), 1.99 (3H, s, $\underline{CH_3}$CO).
ESI-MS: *m/z* 746.97 $[M+Na]^+$; *m/z* 723.58 $[M-H]^-$.

7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5,8-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (**8**), ^{1}H NMR (400 MHz, MeOD) δ: 8.00 (2H, d, J = 8.1 Hz, H2'/H-6'), 6.94 (2H, d, J = 8.3 Hz, H-3'/H-5'), 6.79 (1H, s, H-6), 6.55 (1H, s, H-3), 5.23 (1H, d, J = 7.8 Hz, H-1''), 4.98 (1H, d, J = 8.0 Hz, H-1'''), 1.99 (3H, s, $\underline{CH_3}$CO).
ESI-MS: *m/z* 689.20 $[M+Na]^+$; *m/z* 665.26 $[M-H]^-$.

bartsioside (12), ^{1}H NMR (400 MHz, MeOD) δ: 6.20 (1H, dd, J = 6.3, 2.3 Hz, H-3), 5.85 (1H, d, J = 1.7 Hz, H-7), 5.40 (1H, d, J = 5.2 Hz, H-1), 4.98 (1H, d, J = 6.2 Hz, H-4), 4.24 (2H, br d, J = 10.0 Hz, H-10), 3.16 (2H, m, H-5/H-9), 2.84 – 2.70 (1H, m, H-6a), 2.24 – 2.14 (1H, m, H-6b).
ESI-MS: *m/z* 353.14 $[M+Na]^+$

3. RESULTS AND DISCUSSION

The study of the ethanolic extract of the fresh aerial parts of *S. romana* led to the isolation and the identification of twelve compounds namely (*E*)-phytol (**1**), apigenin 7-*O*-(6''-*O*-acetyl-β-D-glucopyranoside) (**2**), 7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (**3**), 7-{[2-*O*-(6-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy}-2-(3,4-dihydroxyphenyl)-5-hydroxy-4*H*-1-benzopyran-4-one (**4**), 7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (**5**), 7-{[2-*O*-β-D-allopyranosyl-β-D-glucopyranosyl]oxy}-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one (**6**), 7-{[6-*O*-acetyl-2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5,8-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (**7**), 7-{[2-*O*-(6-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranosyl]oxy}-5,8-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (**8**), 6-deoxyharpagide (**9**), harpagide (**10**), ajugoside (**11**) and bartsioside (**12**).

Compound (**1**) is a very common acyclic diterpene alcohol which is part of the chlorophyll structure and is also endowed with interesting biological activities i.e. antiradical, antimicrobial and cytotoxic [21-23]. The presence of (**1**) can explain some of the traditional uses of this species as antibacterial.

Compounds from (**2**) to (**6**) are glycosidic-flavones derivatives of apigenin and luteolin. In particular compounds (**3**), (**4**), (**5**) and (**6**) are diglucosides containing an allose residue in the structure and were already evidenced in some species of the genus such as *S. scardica* Griseb*., S. grandiflora* Salzm. ex Benth*., S. raeseri* Boiss. & Heldr*., S. taurica* Steph. ex Willd*., S. syriaca* L., *S. perfoliata* L., as well as in near species such as *Stachys aegyptiaca* Pers. and *St. anisochila* Vis. & Pancic [14-16,24-27]. These compounds were reported here for the first time in *S. romana*. On the other hand compound (**2**) was evidenced for the first time as a constituent of *Sideritis*, and also in *Lamiaceae* family. Previously, compound (**2**) has been evidenced in few species of Asteraceae such as *Matricaria chamomilla* L. [28] and *Chrysanthemum morifolium* Ramat. [29] and in an Euphorbiaceae species as *Mallotus apelta* (Lour.) Muell.-Arg. [30], thus resulting a quite rare compound. From a taxonomic standpoint it is worth to note that *Lamiaceae* and Asteraceae are families comprised in Asterales order while Euphorbiaceae are comprised in Malpighiales. Acetylated flavonoids derivatives of apigenin and luteolin containing allose have been previously isolated from species comprised in Ajugoideae subfamily such as *Ajuga reptans* and *A. genevensis* [31]. Among flavonoids, isoscutellarein and hypolaetin derivatives of chemotaxonomic relevance (**7** and **8**) were evidenced during this study. In fact, mono and diacetylated isoscutellarein derivatives containing allose have been recently reported for this genus [32] and are traditionally considered as further chemotaxonomic markers (besides iridoids) since their occurrence seems to be restricted to some related genera in the *Lamiaceae* like *Pogostemon*, *Galeopsis, Stachys* and *Sideritis* itself [33].

Considering the ethnomedicinal uses of this species, the presence of a considerable amount of these flavonoid derivatives of apigenin and luteolin, might perhaps justify its traditional use as a herbal tea because apigenin and luteolin glycosides are universally known to exert a strong anxiolytic action [34]. More recently, phenolics of *Sideritis* proved also to possess cytotoxic action toward several cancer lines [35-37] as well as spasmolytic [38] and a potent antioxidant activity [39-41] which resulted comparable to those exerted by *Camellia sinensis* [42]. Moreover, the hypolaetin and isoscutellarein derivatives are endowed with a strong antioxidant and anticholinesterase activity which may result in a neuroprotective effect in the Alzheimer disease [43]. The presence of flavonoids in diglycosidic form improves their solubility in water, the solvent used for infusions, and at the same time their bioavailability. Several analytical studies were conducted to evaluate the phenolic content of mountain teas [5,39,44] and also the urinary excretion of metabolites has been carried out in recent times [45], as a demonstration of the high interest on *Sideritis* species.

Lastly compounds (**9-12**) are iridoids. Iridoids are considered chemotaxonomic markers in *Lamiaceae*, and in particular harpagide and its derivatives. harpagide (**10**) was already evidenced in *Sideritis* [46], while its derivative (**9**) was recognized for the first time as a constituent of *S. romana* as well as in the genus itself. This molecular trait may be characteristic of the studied species since in *Sideritis* were usually

found aucubin derivatives, melittoside or its acylated derivatives: melittoside and 5-allosyloxy-aucubin were found in *Sideritis italica* (Mill.) Greuter et Burter [32], melittoside, 10-*O*-(*E*)-feruloylmelittoside, 10-*O*-(*E*)-*p*-coumaroylmelittoside were evidenced in *S. trojana* Bornm. [47], the latter compound was also recognized in *S. lanata* L. [48]. Harpagide (**10**) is a widespread compound in several species of the *Lamiaceae* while its derivative (**9**) is less common having been evidenced before only in few species such as *Galeopsis tetrahit* L. and *G. pubescens* Besser [17] and *Ajuga iva* (L.) Schreb [49]. It is worth to note that *Galeopsis* genus is comprised in the Lamioideae subfamily of *Lamiaceae* just like *Sideritis* genus, while *Ajuga* is comprised in a different subfamily, Ajugoideae. The presence of (**9**) in *S. romana*, together with the presence of acetylated allosyl flavonoids with apigenin and luteolin aglicones backbone, is a further similarity with the genus *Ajuga,* even if these two genus are comprised in different subfamilies. In fact, there are several reports on isolation of iridoids peculiar of *Ajuga* also in *Sideritis* species: ajugol and/or its derivative ajugoside (**11**), recognized also in the present study, were found in several species of this genus such as *S. montana* L., *S. scardica* Griseb.*, S. syriaca* L. [50], *S. perfoliata* L. subsp. *perfoliata* (syn. of *Sideritis perfoliata* L.) [51], *S. libanotica* Labill. subsp. *linearis* (Bentham) Bornm [52] and *S. lycia* Boiss. & Heldr [53]. The presence of common compounds between these two genera, may have chemosystematic implications, revealing a more strict proximity between themselves. It is worth to note that bartsioside (**12**) was found in *Sideritis* and, more in general, in *Lamiaceae* for the first time in this study. The presence of this compound, even if in little amount, is quite unusual since it is mainly recognized from species traditionally comprised in Scrophulariaceae [20,54,55] (the majority of these species have been recently moved in other families [56]) and Plantaginaceae [57]. Nevertheless, this is not unexpected from a biogenetic point of view since bartsioside is formally the 6-deoxyderivative of aucubin and several *Sideritis* showed the presence of iridoids related to aucubin. Moreover, this species showed also the presence of (**9**), an additional 6-deoxylated iridoid. The presence of (**6**) and (**12**) may be an evidence of a metabolic tendency toward the biosynthesis of deoxy-derivative.

The bioactivities of iridoids (antibacterial, anti-inflammatory, and antiviral activities) has been largely studied, in particular the anti-inflammatory one [58-60]. Also the structure-activity relationship on the basis of the different patterns of substitution (hydroxylation, unsaturation, and acylation) on the iridoid skeleton has been carried out [61]. Accordingly with the last study, the presence of an olefinic bond at C_7-C_8 (aucubin derivative) is one of the most positive character for activity and aucubin derivatives have been found in *Sideritis* [32,47,48]. Among the iridoids identified in *S. romana* bartsioside, presenting a C_7-C_8 unsaturation, may be one of the major responsible of the anti-inflammatory action. Most probably, iridoids are the main responsible of the anti-inflammatory effect also in other *Sideritis* species, even if other active constituents like flavonoids, largely represented in this species, may have obviously a synergistic or modulating action.

4. CONCLUSIONS

In conclusion, during our work on this plant we isolated and identified several different compounds of systematic importance. Among these, four glycosidic apigenin and luteolin derivatives were evidenced besides isoscutellarein and hypolaetin derivatives which characterize the species of this genus. Compound (**2**) resulted a novelty in the studied species as well as in the genus and given its rarity, it may be a suitable marker at a specific level, while compounds from (**3**) to (**8**) were already evidenced in other *Lamiaceae* as *Sideritis* and *Ajuga*. In addition to this, it's worth to note the finding of 6-deoxyharpagide (**9**), harpagide (**10**) and ajugoside (**11**) which, though they are not new in the genus, were reported for the first time in this species, as well as (**12**) never reported before in *Lamiaceae*. The presence of (**9**) is an additional similarity with some *Ajuga* species and, together with the presence of the isolated flavonoids, may be an evidence of a more strict proximity among these genera of *Lamiaceae* even if comprised in two different subfamilies. Moreover, the presence of a huge amount of flavonoids and iridoids, together with (*E*)-phytol, may support the traditional uses of this plant by exerting a wide range of bioactivities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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